CASE: **LA0112 NP**

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Burton Rodney
Type or print name

Signature

9/26/2006
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
TIMUR GUNGOR, ET AL.
APPLICATION NO: **10/775,742**
FILED: **02/10/2004**
FOR: **NOVEL THIAZOLIDINE COMPOUNDS AS CALCIUM SENSING RECEPTOR MODULATORS**

ART UNIT: **1626**
EXAMINER: **STOCKTON, LAURA LYNNE**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PRIOR INVENTION OF RAMAKRISHNA SEETHALA TO OVERCOME CITED U.S. PATENT NO. 6,673,821

To the Commissioner for Patents and Trademarks:

1. This Declaration is to establish reduction to practice of the invention in this application at a date prior to October 22, 2001, that is the filing date of U.S. application Serial No. 10/007,342, now U.S. Patent No. 6,673,821.

2. Ramakrishna Seethala declares as follows.

3. He was awarded a Ph.D. in Biochemistry.

4. He has been employed by Bristol-Myers Squibb Company, the assignee of the subject application as a biologist, for 16 years, about four years of which as a supervisor in the Department of Metabolic Diseases, Age Related Diseases and Bone Biology.

5. That prior to October 22, 2001, a sample of the compound of Example 1 (identified as BMS 515,832) of the subject application was received by Dr. Ramakrishna Seethala for testing of such compound as a modulator of the calcium sensing receptor.

6. Prior to October 22, 2001, he was requested to test the compound of Example 1 (BMS 515,832) of the subject application for its activity as a modulator of the calcium sensing receptor.

7. Prior to October 22, 2001, he requested Zhengping Ma, a biologist working under his supervision, to carry out experiments to test the compound of Example 1 (BMS 515,832) for its activity as a modulator of the calcium sensing receptor.

8. The calcium receptor inhibitor assay employed for such testing was Protocol CaR_H_IC50 which is as follows:

Calcium Receptor Inhibitor Assay Methods:

Inhibition of intracellular calcium:

Calcilytic activity was measured in human TT cells (ATCC No. CRL-1083) by determining the IC50 of the test compound for blocking increases in intracellular Ca2+ by extracellular Ca2+ (as agonist of the receptor). Intracellular Ca2+ was measured using Fluo3,AM (Molecular probes, # F-1242) as indicator dye. Intracellular Ca2+ increase was measured with extracellular Ca2+ from 0.5 to 5 mM in Fluoescence Imaging Plate Reader (FLIPR) (Molecular Devices).

The Ca2+ receptor inhibitor assay procedure is as follows: TT cells were maintained in T-150 flasks in cell growth medium (F-12K Nutrition Media (Gibco 211270-022) with 10% heat

inactivated FBS, and 1x Glutamax) in 5% CO_2:95% air at 37°C to 90% confluency. The medium was removed, the cell monolayer was washed with phosphate buffered saline (PBS), incubated with 0.05% trypsin at 37°C for 2 minutes and the cells were dispensed by agitation. Cells from 2 flasks were pooled and centrifuged (200xg). The cell pellet was suspended in cell growth medium. Cells were plated 30,000 cells/well for 2 days, or 24,000 cells/well for 3 days in 96-well black view plates (Falcon, VWR#624-06-468) and incubated in 5% CO_2:95% air at 37°C. Cell medium was aspirated, and cells were loaded with Fluo3 (Molecular Probes, 50 µg dissolved in 25 µl DMSO, 50 µl 20% Pluronic Acid) in base buffer (10 mM HEPES buffer containing 1x Hank's salt, 0.1% BSA, 0.05% D-glucose, 0.8 mM $CaCl_2$) or 1 hour in a 37°C incubator. After incubation, loading buffer was aspirated and 120 ul/well base buffer was added.

Drug plates were prepared in base buffer and loaded into FLIPR.30 ul from drug plate was added to the cell assay plate and fluorescence signals were read in FLIPR. Drug plate was replaced with $CaCl_2$ plate in FLIPR plate draw and 30 ul $CaCl_2$ (1.7 mM final for IC50s, or 2.0 mM for screening) was added into cell plate by FLIPR. The fluorescence signal was measured by reading at 1 second intervals for 30 seconds and at 3 second intervals for the next 150 seconds. Calcilytic activity of the compounds was measured by their ability to block, in a concentration dependent manner (half-log concentrations in triplicate), the intracellular Ca2+ level by extracellular 1.7 mM Ca2+. The data was processed by ActivityBase (IDDBS) and the IC50 values are determined by protocols developed.

9. Prior to October 22, 2001, Zhengping Ma, working under the supervision of Dr. Ramakrishna Seethala, conducted experiments wherein the Example 1 compound identified as BMS 515,832 was tested for its activity as a modulator of the calcium sensing receptor (Ca R response in TT human cells) and recorded the test results in Notebook No. 49513 pages 079-081, 083 and 084. (ATTACHMENTS K through Q)

10. A summary of the test results obtained by Zhengping Ma prior to October 22, 2001, and recorded in Notebook No. 49513, pages 079-081, 083 and 084, working under the supervision of Dr. Ramakrishna Seethala, is set out in a summary sheet (ATTACHMENT R) prepared after October 22, 2001. As seen in the summary sheet, the Example 1 compound was tested as per the

above protocol for its activity as a modulator of the calcium sensing receptor and was found to have such activity.

11. The actual dates of Experiments regarding the preparation of the Example 1 compound recorded in Notebook No. 49,513, pages 079-081, 083 and 084 were carried out and the dates of signing by Zhengping Ma and witnessing by Yong Quan, were all prior to October 22, 2001, but have been obliterated.

12. Portions of Notebook No. 49,513, pages 079-081, 083 and 084 have been obliterated because they were not relevant to the conception and reduction to practice of the present invention as it concerns Example 1 compound.

13. The above clearly establishes conception and reduction to practice of the invention covered by the relevant claims of the subject patent application (vis-à-vis U.S. Patent No. 6,673,821) prior to October 22, 2001.

14. This Declaration is submitted prior to Final Rejection.

15. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of application Serial No. 10/775,742 or any patent issued thereon.

Date: 9/20/2006

RAMAKRISHNA SEETHALA

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

BRISTOL-MYERS SQUIBB

NOTEBOOK No. 49513

Assigned to Shengping Ma

Subject

Department Name Aging Research

Department Number 800 1602

Date Assigned

Date Completed

Pages Completed from 021 to 210

Continued from Notebook Number 44423

Continued in Notebook Number 51731

ATTACHMENT K

TABLE OF CONTENTS

PROJECT OR EXPERIMENT NO.	PRODUCT OR SUBSTANCE	STUDY PERFORMED OR OBJECTIVE	PAGES
	IC50s: BMS-515832-02-002, 280429, 280587	CaR response in T cells	49513-079 → 49513-088

CaR response in TT cells

TT cells plated o... at 24,000 cells/well used (see also 49513-068)
0.8 mM Ca^{++} basal, 1.7 mM Ca^{++} stimulation.
See also 44676-072 for basic protocol

Plate 1

BMS-515832:02-002 (uM) (A1:C6)					synthesis	
	1	2	3	4	5	6
A	1.0000	0.3333	0.1111	0.0370	0.0123	0.0041
B	1.0000	0.3333	0.1111	0.0370	0.0123	0.0041
C	1.0000	0.3333	0.1111	0.0370	0.0123	0.0041

ATTACHMENT M

SIGNED DATE WITNESSED AND UNDERSTOOD BY: DATE

Signal Test

Plate 1 ZMCa072601a_n0

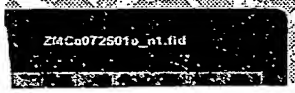
Minimum 9045.6 -16.47% Maximum 12823.2

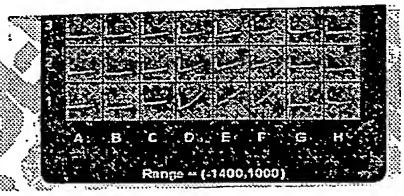
Average 10829.4 STDEV: 738.3

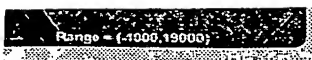
	1	2	3	4	5	6
A	11194.4	10826.4	10314.4	10444.0	10192.8	9940.0
B	10764.0	11074.4	10508.0	9893.6	9832.8	9298.4
C	9922.4	11116.0	10592.8	10845.6	10831.2	10120.8

ATTACHMENT IV

continued







ATTACHMENT O

WITNESSED AND UNDERSTOOD BY:

DATE

File = D:\mazh\ZM\Ca072601a_n1.fid Plate 1

Statistic = Max - Min

Start Sample = 13 End Sample = 45

Positive Scaling = On Negative Correction = Off

Bias Value Subtract = On Spatial Uniformity Correction = On

Bias Sample = 1

	A	B	C	D	E	F	G	H
1	3.79	4.98	2.39	43.82	15.29	26.01	2.4	3.96
2	4.56	4.47	3.85	60.51	45.13	65.27	4.92	3.28
3	11.06	12.77	17.3	95.26	62.57	82.4	20.93	11.91

ATTACHMENT P

SIGNED Zhenyi Ma DATE

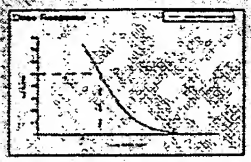
WITNESSED AND UNDERSTOOD BY: DATE

Continued

Test Occasion ID: MDCaR010726-1
Protocol ID: CaR_HTRF_IC50
Study ID: CaR
User ID: Zhengping Ma

Plate 1

Compound ID	Conc (μM)	% TL1	% TL2	% TL3	Avg %TL	StDev	IC50
BMS-515632:02-002	1.000	3.79	4.98	2.39	3.72	1.30	0.024921
	0.333	4.56	4.47	3.85	4.29	0.39	Hill
	0.111	11.06	12.77	17.3	13.71	3.22	1.15
	0.037	32.3	43.99	44.12	40.14	6.79	
	0.012	56.61	48.98	99.99	68.53	27.51	
	0.004	41.18	61.95	37.58	46.90	13.16	



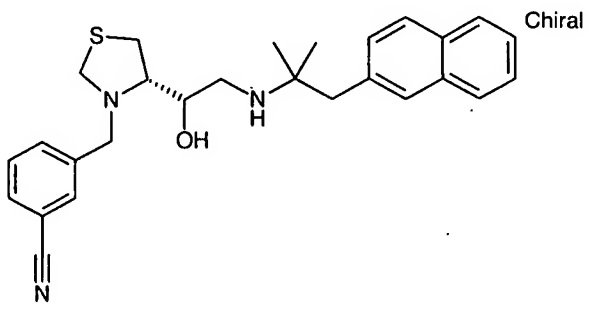
ATTACHMENT Q

SIGNED Zhengping Ma DATE

WITNESSED AND UNDERSTOOD BY: DATE

Pcris.db

2D | 3D | Biology | CTR | HTS | Inventory | Library | Program | Property | Reaction | Reagent | Select | Info



BMS #	Form	Lot	Molecular Wt	Formula Wt
BMS-515832	02	002	445.63	518.55

Formula	Appearance	Location	Project #
$C_{27}H_{31}N_3OS$	off-white powder	HW	08007

Submitter	Chemist's Notebook	Amount (MG)	Purity
Chen,Ying	48255-108-20	82.0	98.1

Date	Molname
	isomer B

Protocol	Version	Biologist	Biologist's Notebook
CaR_H_IC50	1	MAZH	

Protocol Name	Protocol User Group	PWG List Name
Ca+ Sensitive Receptor, Human species, Dose Response assay	MD_PPAR	

Parameter	Assoc Value	Result Comments
RECEPTOR	Ca SENSITIVE	
SPECIES	HUMAN	

Library Name

Alliance ID

Test Occasion	Test Occasion Notes	Parent_Study
MDCaR010726-1		
MDCaR010726-1		
MDCaR010726-1		
MDCaR010809		
MDCaR010816		
MDCaR010816		

Condition 1	Condition 2	Condition 3	Condition 4	
				Name
				NValue
				Units

Study	Protocol	V	Type	AValue	< / >	NValue	Units	C	C 1 AValue	C 2 AValue	C 3 AValue	C 4 AValue	Date
CaR	CaR_H_IC50	1	HILL	-1.15		-1.1500							
CaR	CaR_H_IC50	1	IC50	0.024921		0.0249	uM						
CaR	CaR_H_IC50	1	P_CONTROL	4.29		4.2900			0.33				

ATTACHMENT R